derivative selected from the group consisting of:
an ester of hyaluronic acid wherein part or all of the carboxylic groups of said hyaluronic acid are esterified with alcohols of the aliphatic, aromatic , arylaliphatic, cycloaliphatic series;
an autocrossliked ester of hyaluronic acid wherein part or all of the carboxylic moieties of said hyaluronic acid are esterified with the alcoholic groups of the same or a different hyaluronic acid chain;
a hemiester of succinic acid or a heavy metal salt of the hemiester of succinic acid with hyaluronic acid or with a hylauronic acid ester having part or all of the carboxy groups of hyaluronic acid esterified with an alcohol of aliphatic, aromatic , arylaliphatic, cycloaliphatic series.

101. (new) A biological material comprising:
autologous or homologous cells belonging to at least one cell type selected from the group consisting of endothelial cells, glandular cells, skin adnexa and germinative cells of hair bulbs, a biocompatible three-dimensional matrix, on which said cells are seeded and grown, said matrix comprising an ester of hyaluronic acid wherein part or all of the carboxylic groups of said hyaluronic acid are esterified with alcohols of the aliphatic, aromatic , arylaliphatic, cycloaliphatic series.

Kindly cancel claims 87-121.

**REMARKS**

Applicant wishes to bring to the Examiner's attention U.S. Patent Nos. 4,851,521 and 5,202,431, which are U.S. patents corresponding to EP 0216453, which disclose the hyaluronic acid derivatives of Class A. EP 0216453 was cited on the Information Disclosure Statement filed June 5, 2000.

On pages 2-5 and 16-17 of the Office Action, the Examiner rejected claims 87, 90, 91, 93, 94, 99, 103-105, 107-109, 112,

113, 115, 116 and 121 under 35 U.S.C. § 102(b) as being anticipated by Bellini et al (WO 96/37519) (hereinafter "Bellini").

Reconsideration is respectfully requested.

The term "hyaluronic acid" has a clear meaning to a person skilled in the art, namely it refers to a linear polysaccharide formed by alternating N-acetyl-D-glucosamine and D-glucuronic acid units (see enclosed Merck Index monograph 4675 (Reference 1). As is clearly stated by Bellini in the enclosed declaration under 37 CFR § 1.132, a person skilled in the art would understand the phrase: "ester of hyaluronic acid wherein part or all of the carboxylic groups are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series" as referring to a linear polysaccharide, in which some or all of the carboxy groups are esterified with the indicated alcohols. It is clear that the above definition of the compounds of class A does not encompass the product of Bellini, which is a cross-linked derivative of a hyaluronic acid ester.

To further stress this point, the Applicant has cited supra U.S. Patent Nos. 4,851,521 and 5,202,431, which are the U.S. patents that correspond to previously cited European patent EP 0216453. It is noted that said patents do not refer in any way to cross linked derivatives of hyaluronic acid such as those of Bellini. This is also confirmed in Bellini where it is disclosed that:

The esters of hyaluronic acid disclosed in U.S. 4,851,521 are the precursors of the polysaccharide hydrogel material of Bellini (page 2, lines 2-13; page 3, line 24 to page 4, line 5) which are subjected to UV, b and g polymerization to give a cross linked product;

The esters of hyaluronic acid disclosed in U.S. 4,851,521 have completely different physical structures as compared to the hydrogel material of Bellini (page 3, lines 12-23).

Although the Examiner has not raised any issue regarding the novelty of a biological material comprising the hyaluronic acid derivatives of Class B, we would like to point out that the attached declaration clearly shows that these polymers have a

completely different chemical structure from that of the hyaluronic acid derivatives of Bellini.

Therefore, in view of the above and the arguments already set forth in our previous amendment, the biological material as claimed in originally filed claims 87 and 109, now claims 122 and 144, is not anticipated by Bellini.

For the foregoing reasons it follows that the methods of carrying out human and veterinary surgery as claimed in originally filed claim 99, now claim 134, which make use of the biological material of the present invention, are unanticipated by Bellini.

On pages 5-12 of the Office Action the Examiner rejected claims 87, 94-95, 98, 109, 116-117 and 120 under 35 U.S.C. § 103(a) over Bellini et al. in view of Cialdi et al, U.S. Patent No. 6,027,741 (hereinafter "Cialdi").

Reconsideration is respectfully requested.

The Examiner is urging that Bellini discloses a cross-linked hyaluronic acid derivative and teaches that this can be used as a support for the growth of a plethora of cells among which endothelial, Kupfer's and Langerhans cells are cited. However, the Applicants would like to point out that Bellini does not teach in any way that the material described therein is a <u>better support</u> for the growth and proliferation of fragile cells compared to other materials usually employed as substrates for anchorage dependent cells. On the contrary, the fact that fragile cells are listed together with cells that may be easily cultured would induce a person skilled in the art to believe that the material of Bellini, when used as a support for fragile cells, would not show any particular advantage over other support materials known in the art.

Additionally, as discussed above, the hyaluronic acid derivative of Bellini not only differs chemically from that disclosed in the present application, but it is also characterized by a completely different physical structure. In particular, Bellini's material shows better physical properties such as a more compact three dimensional structure and a greater mechanical resistance. Bellini teaches the use of a different

substrate than the substrate that is contemplated by the present invention, and for this reason does not make the claimed subject matter obvious.

As discussed supra, Bellini's teaching is per se deficient with regard to the use of the disclosed hyaluronic acid derivative as a support for fragile cells. Moreover, Bellini could not suggest to a person skilled in the art, the use of a different hyaluronic acid derivative having different physical properties, such as the hylaluronic acid derivative of the present invention as a support for fragile cells in order to improve their proliferative rate and survival times.

Furthermore, the applicant would also like to point out that Bellini does not disclose that the hydrogel material described therein is able to promote proliferation of the cells cited above. In fact, the term "support for the growth of cells" used in Bellini does not mean that such a support promotes the proliferation of cells. Indeed, as it is demonstrated by attached Reference 2 (Molecular Biology of the Cell) and attached Reference 3 (McGraw Hill Dictionary) a cell can also grow in the absence of any proliferation by increasing its protoplasm.

Moreover, Bellini's deficiencies cannot be overcome by combining its teachings with the Cialdi patent.

Cialdi discloses O-sulfated hyaluronic acid derivatives of Class E in the originally filed claims, now Class D. Although Cialdi teaches that such hyaluronic acid derivatives can be obtained in a three dimensional form, it does not suggest their use as substrates for the growth of cells.

Notwithstanding, the Examiner deems that the data obtained in Example 14 (showing that when a sulfated hyaluronic acid derivative is dissolved in the culture medium the endothelial cells exhibit a better proliferation rate) would induce a person skilled in the art to use such material as a support for the growth of endothelial cells. It is the Applicant's opinion that this is clearly a hind sight consideration.

The fact that a particular polysaccharide promotes the proliferation of particular types of cells when dissolved in a culture medium does not provide any information about its

suitability to promote the growth and proliferation of cells when used as a support. It is well known in the art that anchorage dependent cells need to attach and spread out on a support in order to grow and propagate in culture (see attached copy of The Scientist). Therefore, the main requirement that a material must meet to be a suitable support for the growth of a particular type of cell is that such cells must bind or attach strongly to it. The experiment of Example 14 would induce a person skilled in the art to use a medium containing sulfated hyaluronic acid for the growth of endothelial cells in culture. However, it is clear that the experiment of Example 14 does not give to the person skilled in the art any indication about the ability of endothelial cells to attach, grow and proliferate on a support consisting of sulfated hyaluronic acid. Therefore, there is no basis on which to urge that a person skilled in the art would be induced by the teaching of Cialdi to use a sulphated hyaluronic derivative of Class E as a support on which endothelial and other fragile cells can be grown and propagated.

The Applicant would also like to point out that the hyaluronic acid derivative of Cialdi is a sulfated hyaluronic acid or hyaluronic acid ester which is completely different from the cross-linked hyaluronic acid derivative of Bellini. Therefore, there is no suggestion or motivation for one who is skilled in the art to combine the teachings of Cialdi and Bellini which points to the use of a hind sight approach to the consideration of patentability which is clearly improper.

Consequently, it is submitted that the present invention is not obvious over Bellini in view of Cialdi.

Moreover, at page 11 of the Office Action the Examiner points out that although "Bellini et al do not teach explicitly the process of making the hydrogel material support the aforementioned cells" this would have been evident on the "effective filing date of the present application" from the references supplied in the Information Disclosure Statement (hereinafter "IDS").

This argument is improper because the references cited in the IDS do not teach the preparation of biological material which

comprise "fragile" cells that are seeded and grown in a biocompatible three-dimensional matrix, where said matrix is made of a hyaluronic acid derivative selected from groups A, B and D of claim 1.

On pages 12-15 of the Office Action the Examiner rejected claims 87-89, 91-97, 99-105, 107-108 under 35 U.S.C. § 112, first paragraph.

Reconsideration is respectfully requested.

The Examiner states that the term "cellular line" in the above cited claims stands for a group of "cells of standardized, genetically homogenous type" that are able "to divide indefinitely in culture." The Examiner states that at the filing date of the application, with the exception of the HUVEC cell line, cell lines derived from glandular cells, skin adnexa and germinative cells of hair bulbs were unknown. As a result, it is the Examiner's opinion that since the specification does not give any indication on how to prepare such cell lines, the specification does not contain an enabling disclosure for the biological material as claimed.

The Applicant disagrees with the Examiner's definition of the term "cellular line". As shown in the attached Dictionary of Biochemistry and Molecular Biology and Culture of Animal Cells (References 5 and 6 submitted herewith), the term "cell line" stands for "a heterogeneous group of cells that are derived by subculture of a primary culture". On the contrary, what the Examiner indicates as a "cell line" is indeed more precisely indicated as "established or continuous cell line". The Examiner's interpretation of the term "cell line" derives from the fact that this term is sometimes improperly used in the art, for the sake of brevity, to also indicate continuous cell lines.

That the cell lines of the invention are not continuous cell lines is further confirmed by the fact that the HUVEC line, which is one of the cell lines of the invention, can be propagated for a limited number of population doublings (see Umbilical Vein Endothelial Cells - Reference 7).

Furthermore, the Examiner states on page 12 of the Office Action that "[t]he specification does not reasonably provide

enablement for other embodiments of the claims and how to make and use the invention commensurate in scope with the claims". As shown by Bulletin Hosp. Jt. Dis., Tachiban et al. and Atala et al. (References 8, 9 and 10), at the time of filing of the present application, the skilled worker in the art already knew how to use biomaterials comprising different cells derived by subculture of a primary culture for the replacement of diseased or deficient organs and/or tissues.

Therefore, it is apparent that the present specification, when considered in view of the state of the art at the time the present application was filed, provided a skilled worker in the art with sufficient guidance, to enable such an individual to make and use the biological material of the invention.

At page 15 of the Office Action the Examiner rejected claims 87-108 under 35 U.S.C. § 112, second paragraph.

The Examiner maintains that the meaning of the term "homologous" used in the claims is unclear.

The Applicant respectfully disagrees with the Examiner's opinion.

The term "homologous" has a well recognized meaning in the art. In particular, as indicated in the Culture of Animal Cells (Reference 6), this term is defined as "pertaining to genetically dissimilar individuals of the same species; allogenic".

Therefore, based on the preceding information "homologous cellular line", "homologous fibroblasts" and "homologous endothelial cells" are defined as cellular line, fibroblasts or endothelial cells that derive from an individual belonging to the same species as the one in which the biological material is to be implanted.

Based on the above amendments, remarks and enclosed declaration applicant respectfully submits that Claims 121-161 are allowable over the prior art and that the present application is in proper form for allowance. Reconsideration of these rejections is requested in view of this amendment.

For these reasons, it is requested that the grounds for rejection be withdrawn.

An early and favorable action is earnestly solicited.

Respectfully submitted,

James V. Costigan
Registration No: 25,669

MAILING ADDRESS
Hedman & Costigan, P.C.
1185 Avenue of the Americas
New York, NY 10036
(212) 302-8989

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